IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Arne HOLMGREN et al.

Group Art Unit : 1621

Appl. No : 09/926,218
(National Stage of PCT/JP00/02076)

Examiner : Katakam

I.A. Filed : March 31, 2000

For : SUBSTRATE FOR THIOREDOXIN REDUCTASE

SUPPLEMENTAL REPLY UNDER 37 C.F.R. 1.116
IN RESPONSE TO DECEMBER 24, 2008 OFFICE ACTION

Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window, Mail Stop **AF**
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir :

This is in response to the Final Office Action mailed December 24, 2008, which sets a three-month shortened statutory period for response until March 24, 2009, as well as the Advisory Action mailed April 29, 2009.

Applicants hereby request an extension of time for three months until June 24, 2009, and are concurrently filing a formal Request for Extension of Time for three months accompanied by the government fee. If for any reason the formal request for extension of time is not associated with this reply or any fees are required to maintain the pendency of this application, including any extension of time fees, such as any extension of time for entry of an Examiner's Amendment, and claim fees, this is an express request for any required extension of time and authorization to charge any necessary fee to Deposit Account No. 19-0089.

Applicants are submitting a Notice of Appeal on even date herewith.

Entry of this response with reconsideration and withdrawal of the rejection of record are respectfully requested.

Remarks begin on page 3 of this paper.

REMARKS

Upon entry of this response, claims 13-19 and 26-28 will remain pending, with claims 13, 15 and 17-19 being independent claims.

Reconsideration and allowance of the application are respectfully requested.

Completion of Record

Applicants are submitting on even date herewith a Completion of Record wherein a European Office Action in connection with the counterpart European application is submitted. This Office Action once again references Engman, which was cited in the Supplementary Partial European Search Report as made of record in the Fourth Supplemental Information Disclosure Statement, filed May 13, 2008.

Response To Rejection

Applicants once again note that the following rejection is set forth in the Final Office Action:

Claims 13-19 and 26-28 are rejected under 35 U.S.C. 102(b) as being anticipated by Engman et al. (hereinafter "Engman"), "Diaryl Chalcogenides as Selective Inhibitors of Thioredoxin Reductase and Potential Antitumor Agents", Anticancer Research, Helenic Anticancer Institute, Anthens, GR, Vol. 17, No. 6D, 1997, pp. 4599-4605.

Applicants submit that the rejection is without appropriate basis in that (as previously argued by Applicants throughout the lengthy prosecution of this application) Engman discloses that ebselen was found to be an inhibitor of human thioredoxin reductase. Engman does not disclose the use of ebselen as a substrate for thioredoxin reductase. See, for example, the abstract of Engman. Engman

merely concludes that ebselen is an inhibitor of thioredoxin reductase. As stated in Engman's abstract, ebselen is not a competitive inhibitor for thioredoxin, but for thioredoxin reductase. Moreover, Engman uses conditions in his experiments apparently based upon his understanding that ebselen is a competitive inhibitor for thioredoxin reductase and not a substrate for thioredoxin reductase. These conditions would not appear to provide the results as asserted in the Final Office Action and in the Advisory Action, especially in view of the excess amount of insulin included in the experiments.

With regard to the above, Applicants once again respectfully point out that the Final Office Action includes a number of misstatements in support of the rejection that are not clarified in the Advisory Action. Therefore, reconsideration of Applicants' claimed subject matter with correction of the misstatements is once again requested along with clarification of the rejection, if the rejection is maintained. In this regard, because the rejection is unclear, especially in view of misstatements in the Final Office Action, Applicants submit that the rejection should be withdrawn, and the application allowed. **Moreover, if the rejection is clarified, the finality of the Office Action should be withdrawn to afford Applicants an opportunity to respond to any clarified rejection.**

In particular, Applicants once again point out that the Final Office Action makes an assertion, on page 4, in Response to Arguments, that "Please note that a competitive inhibitor is also a substrate." In fact, in the Advisory Action, the Examiner contends that, ""Please note that a competitive inhibitor is also a substrate. A competitive inhibitor competes with the substrate for the same binding site on an enzyme." However, substrates and competitive inhibitors are not the same. As the Examiner realizes, a competitive inhibitor competes with a substrate, and the two are mutually exclusive. In this regard, Applicants referenced in their previous response "Competitive

Inhibitors" (which includes animated graphics that do not clearly show in a print out), downloaded March 18, 2009 from http://www-biol.paisley.ac.uk/Kinetics/Chapter_3/chapter3_2.html and "Competitive Inhibition" downloaded March 18, 2009 from http://en.wikipedia.org/wiki/Competitive_inhibitor. It appears that copies of these documents were not submitted with Applicants' previous response. Moreover, the copies of these documents in Applicants' file are dated March 4, 2009. Accordingly, to ensure that the record is complete, Applicants are submitting herewith the following documents for entry in the file:

"Competitive Inhibitors" (which includes animated graphics that do not clearly show in a print out), downloaded March 4, 2009 (2 pages) from http://www-biol.paisley.ac.uk/Kinetics/Chapter_3/chapter3_2.html ; and "Competitive Inhibition" downloaded March 4, 2009 (4 pages) from http://en.wikipedia.org/wiki/Competitive_inhibitor.

With regard to the above, in accordance with MPEP 609, these documents are being submitted in support of Applicants' arguments, and without being listed on a Form PTO-1449. Accordingly, payment of a fee and/or certification is not required.

Moreover, the Final Office Action, contends that selenite is indicated to be a substrate in Engman. However, there is no correlation provided in the Final Office Action as to why ebselen can be considered to be a substrate based upon any disclosure of Engman, especially when Engman discloses that ebselen is an inhibitor.

On the top of page 5 of the Final Office Action and in the Advisory Action, it is asserted that, "More the substrate more production of reduced thioredoxin, which in turn enhances the peroxidase

activity." There is no support provided for this assertion. Moreover, it is not clear what is intended by this assertion. Therefore, if this assertion is once again maintained, the Examiner is requested to provide support. Also, clarification is requested because it appears that the affect would be the opposite, with the result being the oxidation of reduced thioredoxin.

Moreover, the further remarks regarding claims 15 and 16 are not clear. According to Applicants' findings, ebselen is reduced by the enzyme or thioredoxin, and the thioredoxin stimulates reduction of ebselen. There does not appear to be any teaching or suggestion in Engman of Applicants' finding.

Regarding claims 17 and 18, the Response to Arguments on page 5 is also unclear and statements in the Advisory Action are unclear. The response and Advisory Action contend that, "Thioredoxins are electron donors." However, it is the enzyme thioreductase that is the electron donor. Moreover, in contrast to the assertion in the Office Action, Km for thioredoxin and the selenium compound are the same.

Still further, it is contended in the Final Office Action that "...the unreacted selenium compound expected to oxidize the reduced thioredoxin." However, it is not expected because the selenium compound is disclosed in Engman as being an inhibitor.

Again, Applicants point out that Engman was looking at ebselen as an inhibitor, and used conditions wherein ebselen is used as an inhibitor and not as a substrate. Accordingly, Engman does not disclose each and every feature of Applicants' claims including the conditions recited in Applicants' claims. In particular, Engman discloses in his assay, at page 4600, the paragraph bridging the right and left-hand columns, that, "Thioredoxin reductase activity was measured

spectrophotometrically at room temperature by the oxidation of NADPH at 339 nm in the presence of 15μM human recombinant thioredoxin and 1 mg/ml bovine insulin." (Emphasis added.)

Thus, in each of the assays of Engman, **insulin is present**. **Insulin in the assay affects the results, and is included in the assay apparently because Engman was experimenting with ebselen as an inhibitor.** The conditions used by Engman do not appear to be conditions as recited in Applicants' claims that achieve the results recited in Applicants' claims.

The Advisory Action raises issues of inherency; however the Examiner is reminded that for inherency to be present the Examiner has the burden of showing that the result indicated by the Examiner is the necessary result, and not merely a possible result. In re Oelrich, 212 U.S.P.Q. 323 (CCPA 1981); Ex parte Keith et al., 154 U.S.P.Q. 320 (POBA 1966). For example, the fact that a prior art article may inherently have the characteristics of the claimed product is not sufficient. Ex parte Skinner, 2 U.S.P.Q.2d 1788 (BPAI 1986).

As the Board of Patent Appeals and Interferences states in Ex parte Levy, 17 U.S.P.Q.2d 1461, 1463:

> However, the initial burden of establishing a prima facie basis to deny patentability to a claimed invention rests upon the examiner. In re Piasecki, 745 F.2d 1468, 223 USPQ 785 (Fed. Cir. 1984). In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. In re King, 801 F.2d 1324, 231 USPQ 136 (Fed. Cir. 1986); W.L. Gore & Associates, Inc. v. Garlock, Inc., 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983); In re Oelrich , 666 F.2d 578, 212 USPQ 323 (CCPA 1981); In re Wilding, 535 F.2d 631, 190 USPQ 59 (CCPA 1976); Hansgirg v. Kemmer, 102 F.2d 212, 40 USPQ 665 (CCPA 1939).in order for inherency to be present it must be a necessary result, and not merely a possible results. Ex parte Keith and Turnquest, 154 U.S.P.Q. 320 (B.O.A. 1966).

Accordingly, the rejection must establish that under the conditions of the assays disclosed by Engman, including excess insulin, the processes recited by Applicants would

be present so that, as required in an anticipation rejection, each and every feature recited in Applicants' claims is present in Engman.

Expanding upon the above, with the mixture of ingredients used by Engman, including thioredoxin and excess insulin, one having ordinary skill in the art would not expect the reduction of the substrate, as recited in Applicants' independent claim 13. In other words, the conditions that are present in Engman would not appear to inherently reduce the substrate. Engman specifically discloses conditions that are based upon his belief that ebselen is an inhibitor of thioredoxin reductase, and therefore used conditions bases upon that belief. As noted above, such conditions would not appear to inherently provide the reduction of the substrate, such as in view of insulin being present in excess in the assays.

Moreover, lack of an expectation that of a reduction of the substrate under the conditions disclosed by Engman is especially evident in view of the fact that thioredoxin is oxidized very fast in the presence of ebselen.

Regarding independent claim 15, it would appear that mixtures of ingredients as disclosed in the assays of Engman that include excess insulin would not be expected to enhance peroxidase activity, but would be expected to destroy peroxidase.

Regarding independent claim 17, Applicants note that claim 17 recites therein reduced thioredoxin. In contrast, Engman does not disclosed reduced thioredoxin, but discloses thioredoxin. Moreover, thioredoxin would be expected to be present in Engman in view of the excess of insulin in the assays.

Similar arguments to those set forth above also apply to independent claims 18 and 19 as the conditions disclosed by Engman, including the excess insulin, would not appear to inherently provide Applicants' claimed subject matter.

The Advisory Action raises issues regarding that the result does not have to be realized for inherency to be present. However, the rejection must establish that the same processes are present, and for at least the reasons set forth above, the same processes do not appear to be present whereby the assertion of inherency is not without appropriate basis.

Therefore, Engman does not teach each and every feature recited in Applicants' claims. Engman does not constitute anticipatory prior art as asserted in the rejection, because:

(1) Engman does not disclose, as recited in Applicants' independent claim 13, a method for reduction of a substrate with thioredoxin reductase, comprising combining the thioredoxin reductase, the substrate and NADPH *in vitro* under conditions to reduce the substrate, the substrate being as recited in Applicants' claim 13.

(2) Engman does not disclose, as recited in Applicants' independent claim 15, a method of enhancing peroxidase activity of thioredoxin reductase, comprising combining NAPDH, thioredoxin reductase, thioredoxin and a substrate *in vitro* under conditions to enhance peroxidase activity of thioredoxin reductase, the substrate being as recited in Applicants' claim 15.

(3) Engman does not disclose, as recited in Applicants' independent claim 17, a method of oxidizing reduced thioredoxin by a substrate, the method comprising combining reduced thioredoxin and a substrate *in vitro* under conditions to oxidize the reduced thioredoxin with the substrate, the substrate being as recited in Applicants' claim 17.

(4) Engman does not disclose, as recited in Applicants' independent claim 18, a method for reducing a peroxide comprising combining thioredoxin, thioredoxin reductase, NAPDH and a substrate *in vitro* under conditions to reduce the peroxide, the substrate being as recited in Applicants' claim 18.

(5) Engman does not disclose, as recited in Applicants' independent claim 19, a method of preventing peroxidation of a substance comprising combining thioredoxin, thioredoxin reductase and NADPH with a substrate *in vitro* under conditions to prevent peroxidation of the substance, the substrate being as recited in Applicants' claim 19.

The Examiner is reminded that in contrast to the prior art of record, the present invention recognizes and demonstrates that ebselen is a substrate being reduced by NADPH and thioredoxin reductase with a low Km-value meaning that it is a very good substrate undergoing unlimited cycles of oxidation/reduction in the presence of hydrogen peroxide without affecting the activity of the enzyme. The reduced ebselen is called ebselen selenol and has the Se-N bond broken by reduction. The selenol is oxidized back to ebselen by hydrogen peroxide or another peroxide and a new cycle starts. The reaction is ultimately driven by NADPH. Reduced thioredoxin strongly enhances the thioredoxin reductase reaction which is also proven by determination of the rate of reduction of ebselen by reduced thioredoxin using kinetics with tryptophan fluorescence. The result, never seen before, is that ebselen is a very efficient oxidant of reduced thioredoxin.

Accordingly, for at least the reasons set forth above, each of the pending claims is patentable over Engman, and the rejection should be withdrawn.

CONCLUSION

In view of the foregoing, the Examiner is respectfully requested to reconsider and withdraw the rejection of record, and allow each of the pending claims.

Applicants therefore respectfully request that an early indication of allowance of the application be indicated by the mailing of the Notices of Allowance and Allowability.

Should the Examiner have any questions regarding this application, the Examiner is invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,
Arne HOLMGREN et al.

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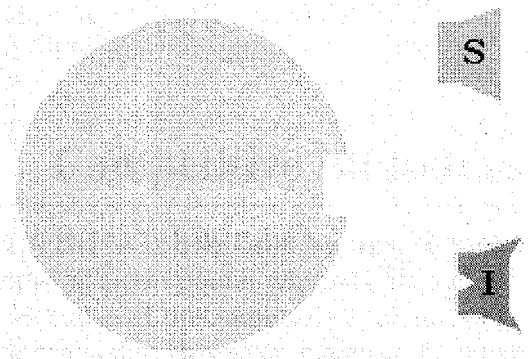
Competitive Inhibitors

Competitive inhibition by active site binding

Classically, a competitive inhibitor is a compound which bears a close structural and chemical similarity to the substrate of the enzyme. Because of this similarity the inhibitor binds to the active site in place of the substrate - a sort of molecular mistake. However, because the substrate and inhibitor are not identical the enzyme is unable to convert the inhibitor into product. The inhibitor simply blocks the active site. While it's there the substrate can't enter and consequently the enzyme can't convert it to product. Similarly, though, if the substrate binds to the active site before the inhibitor, the inhibitor is incapable of binding. The two are said to be *mutually exclusive* - it is impossible for both of them to bind to the active site at the same time.



No inhibitor present - the substrate binds

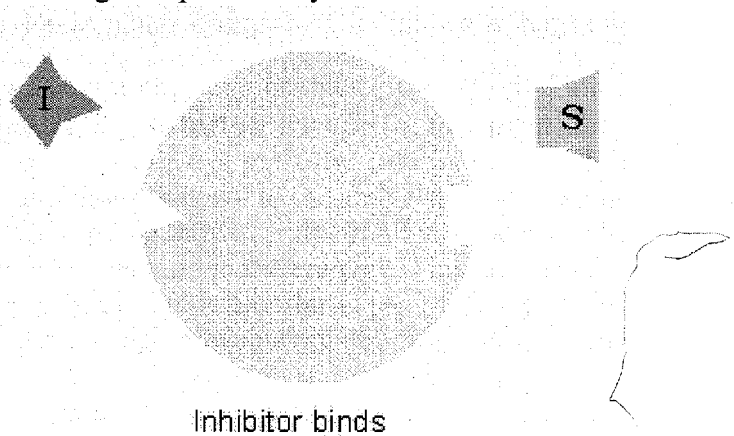
The animated graphic demonstrates this method of inhibition.

Competitive inhibition by conformational change

This is the obvious, and commonest, way for competitive inhibitors to work but it isn't the only way. Another possibility is that the inhibitor binds not to the active site but to an inhibitor binding site which is remote from the active site. On binding, however, the inhibitor causes a change in the three-dimensional shape - a conformation change - in the enzyme. This has the effect of altering the active site such that the substrate can no longer bind to it. Similarly, prior binding of the substrate to the active site causes a change in the inhibitor site which prevents the inhibitor from binding.

Once again it is impossible for both inhibitor and substrate to bind to the enzyme at the same time. They are mutually exclusive.

In this kind of competitive inhibition there is no need for the inhibitor to have any chemical similarity to the substrate, as they are both binding to separate enzyme sites.



Inhibitor binds

The animated graphic shows this mechanism

Kinetics of competitive inhibitors

Since any kind of inhibitor slows down an enzymic reaction it must clearly have an effect on the kinetics. The nature of that effect may be used to distinguish between inhibitor types. The effects of competitive inhibitors on an enzyme's kinetics is discussed on the next page.

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Competitive inhibition

From Wikipedia, the free encyclopedia
(Redirected from Competitive inhibitor)

Competitive inhibition is a form of enzyme inhibition where binding of the inhibitor to the enzyme prevents binding of the substrate and *vice versa*.

Contents

Mechanism

In competitive inhibition, the inhibitor binds to the same active site as the normal enzyme substrate, without undergoing a reaction. The substrate molecule cannot enter the active site while the inhibitor is there, and the inhibitor cannot enter the site when the substrate is there. In this case, the maximum speed of the reaction is unchanged, while the apparent affinity of the substrate to the binding site is decreased (it means: the K_d dissociation constant is apparently increased). The change in K_m (Michaelis-Menten constant) is parallel to the alteration in K_d. Any given competitive inhibitor concentration can be overcome by increasing the substrate concentration in which case the substrate will outcompete the inhibitor in binding to the enzyme.

Equation

$$\text{apparent } K_m = K_m \times \left(1 + \frac{[I]}{K_I}\right)$$

V_{max} remains the same because the presence of the inhibitor can be overcome by higher substrate concentrations.

where K_I is the inhibitors dissociation constant and $[I]$ is the inhibitor concentration.

Derivation

In the simplest case of a single-substrate enzyme obeying Michaelis-Menten kinetics, the typical scheme

E + S <==> ES ---> E + P

is modified to include binding of the inhibitor to the free enzyme:

EI + S <==> E + I + S <==> ES + I --> E + P + I

Note that the inhibitor does not bind to the ES complex and the substrate does not bind to the EI complex. It is generally assumed that this behavior is indicative of both compounds binding at the same site, but that is not strictly necessary. To derive the equation describing the kinetics, first assign microscopic rate constants to each step:

k_1 = E + S --> ES
k_{-1} = ES --> E + S
k_2 = ES --> E + P
k_3 = E + I --> EI
k_{-3} = EI --> E + I

Just as with the derivation of the Michaelis-Menten equation, assume that the system is at steady-state, that is that the concentration of each of the enzyme species is not changing.

$$\frac{dE}{dt} = \frac{d}{dt}ES = \frac{d}{dt}EI = 0.$$

Furthermore, the known total enzyme concentration is $E_T = E + ES + EI$, the velocity is measured under conditions in which the substrate and inhibitor concentrations do not change substantially and an insignificant amount of product has accumulated.

We can therefore set up a system of equations:

eq 1: $E_T = E + ES + EI$

eq 2: $\frac{dE}{dt} = 0 = -k_1 * E * S + k_{-1} * ES + k_2 * ES - k_3 * E * I + k_{-3} * EI$

eq 3: $\frac{dES}{dt} = 0 = k_1 * E * S - k_{-1} * ES - k_2 * ES$

eq 4: $\frac{dEI}{dt} = 0 = k_3 * E * I - k_{-3} * EI$

where S, I and E_T are known. The initial velocity is defined as $v = dP/dt = k_2\ ES$, so we need to define the unknown ES in terms of the knowns S, I and E_T.

From eq 3, we can define E in terms of ES by rearranging to

$$k_1 * E * S = (k_{-1} + k_2) * ES$$

Dividing by $k_1\ S$ gives

$$E = \frac{(k_{-1} + k_2) * ES}{k_1 * S}$$

As in the derivation of the Michaelis-Menten equation, the term $(k_{-1}+k_2)/k_1$ can be replaced by the macroscopic rate constant K_m:

$$\text{eq 5: } E = \frac{K_m * ES}{S}$$

Substituting eq 5 into eq 4, we have

$$0 = \frac{k_3 * I * K_m * ES}{S} - k_{-3} * EI$$

Rearranging, we find that

$$EI = \frac{k_3 * I * K_m * ES}{S * k_{-3}}$$

At this point, we can define the dissociation constant for the inhibitor as $K_i = k_{-3}/k_3$, giving

$$\text{eq 6: } EI = \frac{I * K_m * ES}{S * K_i}$$

At this point, substitute eq 5 and eq 6 into eq 1:

$$E_T = K_m * \frac{ES}{S} + ES + \frac{I * K_m * ES}{S * K_i}$$

Rearranging to solve for ES, we find

$$E_T = ES * (\frac{K_m}{S} + 1 + \frac{I * K_m}{S * K_i}) = ES * \frac{K_m * K_i + S * K_i + I * K_m}{S * K_i}$$

$$\Rightarrow \text{eq 7: } ES = \frac{E_T * S * K_i}{K_m * K_i + S * K_i + I * K_m}$$

Returning to our expression for v, we now have:

$$v = k_2 * ES = \frac{k_2 * E_T * S * K_i}{K_m * K_i + S * K_i + I * K_m}$$

Rearranging and replacing k_2 with k_{cat}, we have

$$v = \frac{k_{cat} * E_T * S}{K_m + S + K_m * (I/K_i)}$$

Finally, we can replace $k_{cat}*E_T$ with V_{max} and combine terms to yield the conventional form:

$$v = \frac{V_{max} * S}{S + K_m * (1 + I/K_i)}$$

See also

- Schild regression for ligand receptor inhibition
- Non-competitive inhibition

Retrieved from "http://en.wikipedia.org/wiki/Competitive_inhibition"
Category: Enzyme kinetics

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